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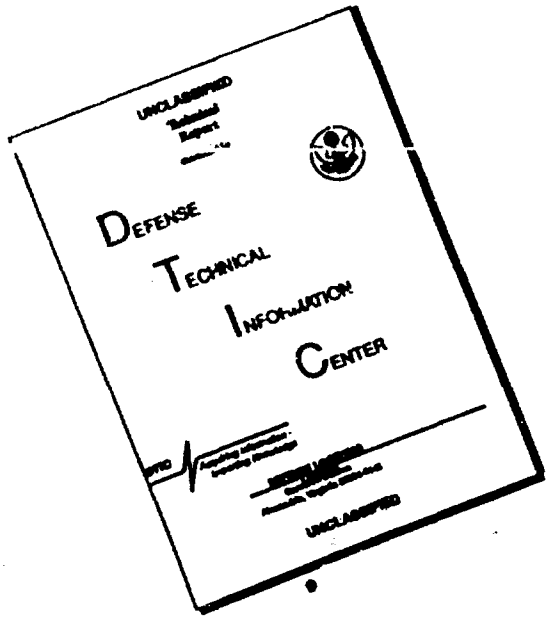

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ON THE TRANSFER OF GENETIC TRAITS BY MEANS OF RNA ISOLATED FROM INFLUENZA VIRUS

[Following is the translation of an article by M. I. Sokolov, R. Ya. Podchernyayeva and L. K. Menshikh, Institute of Virology, imeni D. I. Ivanovskogo AMN USSR, Moscow, published in the Russian-language periodical Voprosy Virusologii (Problems of Virology) No. 2, 1965, pages 139-142. It was submitted on 26 June 1964. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

A number of authors have convincingly demonstrated that the material carrier of heredity in viruses is nucleic acid and that the RNA, isolated from a number of viruses, possesses infectious properties. Contradictory data has been obtained concerning the infectious ability of RNA from the influenza virus [1,3,5,6,9,10,11].

We were not able to isolate infectious RNA from influenza A and A2 viruses by the method of Gierer and Schramm. In the present investigation an attempt is made to clear up the possibility of the transfer of traits with the help of influenza virus RNA during joint cultivation with infectious virus, and also during the administration of the RNA from the 2 viruses.

Materials and Methods

The possibility of the transfer of traits with the help of RNA-containing preparations was studied on surviving tissue from the chorioallantoic membrane of a chick embryo and in a cell culture of chick fibroblasts. The experiments were set up in 2 variations: In one case the RNA of influenza A virus and the infectious virus of A2 influenza were administered to the tissue culture, and in the second case - the RNA of both influenza viruses (A and A2). Controls were tissue cultures, inoculated with infectious virus, RNA, and RNA with RNAase.

The RNA was isolated by the method of cold phenol extraction set up by Gierer and Schramm [7] with certain changes introduced by Portokala [3] and Maassab [9]. For the experiments we used the virus strains of influenza A (WSN) and A2 (F_3). Their properties have been described earlier [4]. As is seen from Figure 1, the RNA preparations had an absorption spectrum with a maximum of 258 mμ, which is typical for nucleic acid. The ratio of the E_{258}/E_{280} and the E_{max}/E_{min} equalled approximately 2, which corresponds to pure high-molecular preparations of RNA. The isolated preparations yielded a negative reaction to protein [8]. As a control we used preparations of RNA, treated with RNAase ("Reanal" firm) in a concentration of 25 μg/ml. Contact with RNAase was for 2 hours at room temperature.

Hybridization, selection and the study of the properties of hybrid variants were carried out by methods which we described earlier, used for the crossing of inactivated and infectious virus [2,4].

The RNA was introduced in a nondiluted form in a volume of 0.2 ml, and the infectious virus - in a dose of 5×10^4 in the same volume. The RNA and virus were administered simultaneously or with an interval of one hour.

Results

We carried out 6 experiments of hybridization on the chorioallantoic membranes of chick embryos and 3 experiments on a culture of chick fibroblasts. Each variant of the experiment and the control was set up in 4 test tubes with tissue culture.

The results of our investigations showed that during the joint cultivation of infectious influenza A2 virus with the RNA of A virus the infectious virus was exposed in the cultural fluid both during cultivation on chorioallantoic membranes and in the tissue culture of chick fibroblasts. With the administration of the RNA from both viruses (A and A2) the infectious virus was detected only after one passage on chick embryos of the cultural fluid taken from test tubes with the chorioallantoic membrane. Following the administration in tissue culture of RNA and RNA together with RNAase (control) the infectious virus was not isolated after 4 passages on chick embryos.

During the joint cultivation of the RNA from influenza A virus with the infectious A2 virus, variants were isolated which possessed the antigenic properties of the latter. A number of biological properties were studied in the bred hybrids.

It was cleared up that following the administration into a tissue culture of RNA, isolated from the inhibitor-resistant strain of the A influenza virus and the infectious inhibitor-sensitive A2 virus strain, inhibitor-resistant strains are formed which preserve the antigenic properties of the A2 influenza virus.

We also studied the transfer of the thermo-resistance of hemagglutinins and infectious properties of the virus at 56°. The results of these experiments are presented in the table, from which it is seen that the thermo-resistance of the infectious properties and the hemagglutinins in the hybrid strains was somewhat higher than in the original ones.

During the hybridization of RNA, isolated from the WSNA-1 strain, which possesses a high activity of multiplication in chick embryos, with the $F_3A2\text{-}1^+$ strain, with a low infecting ability, it was possible to systematically transfer this property to the latter. In 3 out of 5 hybrids the infectious titer rose by 2-3 lg in comparison with the initial strain (Figure 2).

The problem concerning the possibility of the transfer or pathogenic properties for mice was cleared up on the same hybrid strains as when studying the transfer of infecting ability. Following the hybridization of a preparation of RNA from the WSNA-1 strain and the infectious $F_3A2\text{-}1^+$virus, out of the 5 hybrid strains studied 4 acquired the ability to cause the

death of mice following intranasal infection. Virulence in the various hybrid strains was not the same (from $10^{-1.5}$ to $10^{-3.5}$). One of the hybrid strains ($G_5A2\text{-}1^-$) possessed the same virulence ($LD_{50}10^{-3.5}$) as the donor strain from which the RNA was extracted.

For increasing the virulence the variants were passaged through the lungs of mice. Passages of lung suspension were carried out after 48 hours. After 3 passages the $G_1A2\text{-}1^-$ hybrid, which was non-pathogenic for mice, acquired a weak pathogenicity ($LD_{50}10^{-1.25}$), and in another hybrid ($G_3A2\text{-}1^-$) the virulence increased by 2 lg ($LD_{50}10^{-3.3}$). Parallel passages of the strain-recipient on mice did not lead to their acquisition of pathogenic properties.

In 2 hybrid strains ($G_2A2\text{-}1^-$ and $G_3A2\text{-}1^-$) the antigenic and immunogenic properties were checked in immunization experiments on mice. One variant ($G_3A2\text{-}1^-$) possessed the same antigenic and immunogenic activity as the strain ($WSNA\text{-}1^-$) from which the RNA was extracted. Mice, immunized with this variant, were resistant to 7×10^3 LD_{50}/0.05 ml of a virus (strain FM_{30}) which was pathogenic for them. The antibody titer in the sera of the immunized mice was the same as in the donor strain. On the other hand, the second hybrid ($G_2A2\text{-}1^-$), based on immunogenic properties, came close to the strain-recipient $F_3A2\text{-}1^+$.

As was already noted above, following the administration of RNA-containing preparations of type A and A2 influenza virus into a tissue culture, the infectious virus was not isolated in passaging in chick embryos. However, following the administration of RNA from the $WSNA\text{-}1^-$ strain and the RNA from the $F_3A2\text{-}1^+$ strain with an interval of one hour, in 2 tests out of 5 after one passage in chick embryos a virus strain of influenza A was isolated which possessed a number of properties (hemagglutinating activity of chick erythrocytes, resistance to inhibitors of normal horse serum, infectiousness for chick embryos, pathogenicity for mice), characteristic for the $WSNA\text{-}1^-$ strain. At the same time this variant did not cause the death of mice following intracerebral infection and in contrast to the $WSNA\text{-}1^-$ strain possessed a resistance on the part of the hemagglutinins and infectious properties to heating at 56°, characteristic for the $F_3A2\text{-}1^+$ strain.

The titer of antibodies in the sera of mice, immunized with this variant, was the same as in the initial $WSNA\text{-}1^-$ strain, however, their resistance to a pathogenic virus (FM_{30}) was considerably weaker in comparison with that which was caused by the initial $WSNA\text{-}1^-$ strain. Consequently, the type A variant lost neurotropic virulence for mice and acquired a thermo-resistance characteristic for the A2 virus. Along with this, it preserved antigenic activity with a considerable loss of immunogenic properties.

Conclusions

The possibility has been established for the systematic transfer of hemagglutinating capability, inhibitor- and thermo-resistance, and infectious

and immunogenic activity during hybridization with the help of RNA containing preparations, obtained from the viruses of influenza A and A2. In a number of cases the transfer of pathogenicity for mice was observed, and more rarely - the transfer of neurotropic virulence.

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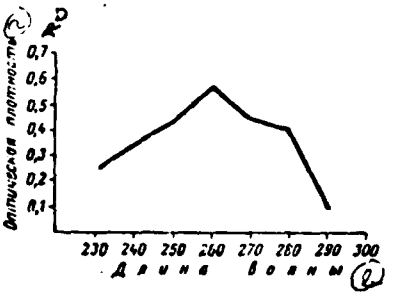

Figure 1. Ultraviolet spectrum of absorption of an RNA preparation from the $F_3A2\text{-}1^+$ strain. a - optical density; b - wave length.

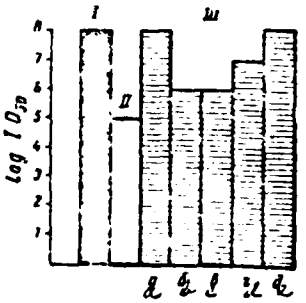

Figure 2. Infectious titers of initial and hybrid strains.
I - donor WSNA-1$^-$; II - recipient $F_3A2\text{-}1^-$; III - hybrids: a - $G_1A2\text{-}1^-$, b - $G_2A2\text{-}1^-$, c - $G_3A2\text{-}1^-$, d - $G_4A2\text{-}1^-$, e - $G_5A2\text{-}1^-$.

Thermo-resistance of hemagglutinins and infectious properties at 56°

Time probe was taken (in minutes)	Strain											
	Donor		Recipient		Hybrids							
	WSNA-1⁻		F_3A2-1^+		G_1A2-1^-		G_2A2-1^-		G_3A2-1^-		G_4A2-1^-	
	RHa	Infectiousness	RHa	Infectiousness	RHa	Infectiousness	RHa	Infectiousness	RHa	Infectiousness	RHa	Infectiousness
5	80	+	40	+	320	+	40	+	320	+	1280	+
10	40	+	40	+	320	+	40	+	320	+	1280	+
20	10	-	40	+	320	+	40	+	160	+	640	+
30	10	-	20	-	160	+	40	+	160	+	640	+
60	--	-	20	-	160	+	40	+	160	-	320	-
120	--	-	20	-	160	-	20	+	80	-	320	-
180	--	-	--	-	---	-	--	-	---	-	---	-

Note: The titers of the hemagglutinins are expressed in reverse values.